20. (Unchanged) The method of claim 17, wherein said attenuated *Streptococcus equi* is in the amount of from about 10^6 to about 10^{10} Colony Forming Unit (CFU).

21. (Unchanged) The method of claim 17, wherein said attenuated *Streptococcus equi* is in the amount of from about 10^7 to about 10^9 Colony Forming Unit (CFU).

REMARKS

Claims 2-21 are pending in the application. Claims 3, 4 and 9 are canceled. Claims 2, 6, 7, 8, 11, 14, 16 and 18 are amended to more clearly define the subject matter of the invention. Therefore after entry of this amendment, claims 2, 5-8, and 10-21 will be pending. No new matter is introduced with these changes. Applicant respectfully requests consideration of the amendments and the following remarks.

Rejection under 35 U.S.C. § 112

Claim 2 has been amended thereby rendering the Section 112 rejection moot.

Rejection under 35 U.S.C. § 103(a)

Claims 2-21 are rejected under 35 U.S.C.§103(a) over U.S. Patent No. 5,183,659 to Timoney, et al. ("Timoney") in view of EP0786515 A1 to Hartford, et al, ("Hartford") and U.S. Patent No. 5,597,807 to Estrada, et al. ("Estrada"). Applicant respectfully traverses the rejection.

Timoney discloses a bacterial vaccine against *Streptococcus equi* (*S. equi*) that stimulated a nasopharyngeal immune response when administered to ponies. The vaccine contained an avirulent strain of *S. equi* (Cornell 709-27) and growth broth

(Todd Hewitt broth). Although Timoney demonstrated that antibodies were formed in the nasopharyngeal mucus of vaccinated animals, this occurred in the absence of the use of any adjuvant in the vaccine. Therefore, all that Timoney teaches is that administration of an avirulent strain of *S. equi* induces an immune response.

Timoney is silent as to adjuvants. Timoney does not suggest or teach one of skill in the art to use adjuvants, nor the desirability of using adjuvants. Certainly, Timoney does not teach or suggest that any adjuvant may stimulate mucosal immunity. Consequently, it is not obvious from Timoney to use saponin in particular to achieve this result. Arguably, even if Timoney suggested the desirability of using an adjuvant to enhance an immunological response, it is not obvious from Timoney that any adjuvant would reasonably be expected to provide an enhanced protective immune response to a particular bacterial antigen. Timoney does not teach or suggest that any adjuvant would enhance the protective effect of an attenuated live *S. equi* vaccine in an animal against infection upon challenge with wild type *S. equi*. Absent any suggestion that an adjuvant, or saponin in particular, has immunostimulatory properties, and that such adjuvant would provide a protective immune response to challenge to disease, one of skill in the art would not be motivated to modify Timoney in order to arrive at the claimed invention.

Hartford and Estrada do not remedy the deficiencies in Timoney. Hartford discloses a vaccine comprising a new strain of *S. equi* which contains a large deletion (about 1kb) in its genome. Hartford lists Quill A (saponin) as only one among many adjuvants that generally might be included in the vaccine, however only specifically teaches that cholera toxin or the *E. coli* heat labile toxin are "specially suitable for mucosal application" (page 3, line 44). Hartford does not teach or suggest, however, that any adjuvant stimulates mucosal immunity. Hartford certainly does not teach or suggest to one of skill in the art that Quill A saponin is an immunostimulatory adjuvant.

4 Estrada also fails to provide the required teachings to render the claimed invention obvious. Estrada teaches that one specific type of saponin, Quinoa saponin, surprisingly stimulated an immune response when administered mucosally. Critically, Estrada does not use *S. equi* or a comparable antigen and thus does not teach or suggest that an immune response may be achieved using the combination of Quinoa saponin and *S. equi* or a comparable bacterial or disease causing antigen. Nor does Estrada teach or suggest that Quinoa saponin provides protection from infection in the face of challenge.

5 Further, Estrada found that Quinoa saponin unexpectedly had different properties than Quil A saponin. Based on these results, one of skill would not have a reasonable expectation that the members of this category of adjuvants would provide an enhanced immune response or protective effect in horses, used alone or in combination with an antigen.

6 Estrada teaches that administration of Quinoa saponin increased IgG and IgA levels, showing only that this particular saponin stimulated an immunological response. However, an immunological response is not predictive of protective immunity in the face of challenge.

7 It is well known to those of skill in the art that no definite correlation exists between the presence of antibodies and protective immunity. Applicant has demonstrated this to be true in the studies described in the specification. For example, on pages 15-16, the results show that even though neither of the vaccinated groups showed a significant difference in ELISA titers throughout the study when compared to the control group (page 16, lines 7-10), a statistically significant difference was seen in the post challenge results in total clinical scores, disease incidences and WBC counts of vaccinated horses compared to the control horses. Thus, a protective effect against severe *S. equi* challenge was demonstrated in the absence of a significant change in antibody titers.

8 If the levels of antibodies are not predictive of protective immunity, then one of ordinary skill in the art would not expect to enhance the protective effect of a vaccine with an adjuvant that had not been shown to be protective, and certainly would have no reasonable expectation that this could be achieved. Thus, the teaching of Estrada that the saponin causes increased absorption through mucosal membranes does not teach or suggest that saponin stimulates protective mucosal immunity when the animal is challenged with an infective organism. Therefore, one of skill in the art could not reasonably predict that this property could be achieved using the Quinoa saponins.

9 As applicant pointed out above, Estrada does not teach the use of *S. equi* or any other bacterial or disease-causing antigen, thus Estrada does not show or suggest that a vaccine containing saponin stimulates a disease specific immune response. Instead, Estrada uses the "model antigens" avidin and cholera toxin which are known adjuvants. Hartford, for example, teaches that cholera toxin is one among many adjuvants known to be non-specific stimulators of the immune system(Hartford page 3, lines 39- 44). Thus, at most, Estrada teaches that a non-specific immunological response to adjuvants is achieved with the administration of saponin. Certainly one of skill could not predict that a vaccine containing saponin would provide protection against contact with a specific disease, based on the teachings of Estrada.

10 Further, based on the teachings of Estrada, one of skill could not reasonably predict that a protective immune response could be achieved using any saponin type. Estrada only teaches the benefits of administering one type of saponin, the Quinoa saponin, and demonstrated that the immunological responses obtained using this adjuvant were unexpectedly different from the results achieved using Quillaja saponin. Applicant again points out that Estrada did not use an antigen that would induce a disease specific response for these studies, rather Estrada used adjuvants known to stimulate non-specific immune responses as model antigens. Estrada discloses that mucosal administration of Quinoa saponin/cholera toxin induced

a higher primary serum IgG and IgA response than that obtained with the administration of Quillaja saponin/cholera toxin (see col. 11, lines 1-8 and Figure 2 and 3). Estrada also shows that Quinoa saponin induced a higher IgA response to the "model antigen" compared to the model antigen alone or Quillaja saponin with model antigen (see col. 11 lines 12-14 and Figure 5). Thus Estrada specifically teaches that Quinoa saponins rather than Quillaja saponins consistently enhance nonspecific immunity and cause increased absorption through mucosal membranes. Estrada indicates that these results are unexpected in view of prior art reports that Quinoa saponins lacked any adjuvant activity. (col. 2, lines 25-27). Clearly, based on these results, one of skill in the art could not reasonably predict the properties of any type of saponin used as an adjuvant.

16 Based on the knowledge that an immune response is not predictive of a protective response, and in view of the limited teachings of Estrada discussed above, one of skill certainly could not predict that the use of Quillaja saponins in a vaccine with attenuated *S. equi* would enhance a specific immunostimulatory protective response to challenge with disease, such as challenge by wild type *S. equi*. Estrada does not supply the suggestion or motivation missing from Hartford and Timoney to use saponin in an *S. equi* vaccine as an immunostimulant. Therefore, the combination of cited references does not render the claimed invention obvious.

17 The Examiner further asserts that Timoney teaches that the mouse model is routinely used to establish the protective effect of vaccines *in horses* (emphasis added). Timoney states that "the mouse has historically been the model for the immunology of *S. equi* infection." (col. 6, lines 30-31). However, all that this teaches is that the mouse model has been used to study the immunological response in mice to potential *S. equi* vaccines. Timoney did not extrapolate the data in mice to conclude or suggest that there would be a similar effect in horses.

18 With regard to the mouse model, Hartford does not teach that this model establishes protective effects of vaccines in horses. Hartford tested the *S. equi*

vaccine in mice and confirmed that the specific bacterial strain tested was attenuated and that it conferred protective immunity *in mice*. Hartford, like Timoney, did not extrapolate or suggest that the results in mice would have a similar effect in horses. Although Hartford did test the vaccine in horses, only the safety of the vaccine was tested. Hartford could only conclude from these tests that the vaccine was safe in horses. One of skill in the art would not reasonably conclude from the teachings of Hartford, without actually performing the tests in horses, that the vaccine would confer protective immunity to horses. The Examiner's conclusion that the vaccine intrinsically would provide protective immunity in horses is not supported by the teachings of the references.

14 It appears that the Examiner has applied hindsight reasoning to the cited combination of references. *Grain Processing Corp. v. American Maize-Products Corp.*, 840F2d 902, 907; 5 USPQ2d 1788,1792 (Fed. Cir. 1988). The Court in Grain Processing held that:

> Care must be taken to avoid hindsight reconstruction by using the patent in suit as a guide through the maze of the prior art references, combining the right references in the right way so as to achieve the results of the claims in suit.

840 F2d. at 902.

15 One of skill in the art would, at best, only be motivated to combine Hartford and Estrada with Timoney based on the present invention because Timoney is silent as to the use of any adjuvants. Applicant submits that it would not have been obvious that any adjuvant would provide the enhanced protective immunological effect demonstrated by the claimed saponin/attenuated *S. equi* vaccine.

16 Applicant further provides evidence, in the form of a Declaration, that it would not be obvious, in view of Timoney, Hartford, and Estrada, either taken alone or in combination, that the use of saponin as an adjuvant with attenuated live *S. equi* would significantly enhance the immunostimulatory effect of the intranasal vaccine.

Applicant submits herewith a Rule 132 Declaration of Hsien-Jue Chu which provides a direct comparison of the efficacy and immunostimulatory properties of the claimed intranasally administered *S. equi*/saponin composition compared to a commercially available intramuscularly administered *S. equi* vaccine. The commercial vaccine contains an enzyme extract of *S. equi* plus a Carbopol-based adjuvant (Strepgard with Havlogen, Bayer Corporation; see Attachment A). The data in the Declaration provide evidence of unexpected properties of the claimed invention over the prior art. The data show that the claimed intranasal *S. equi*/saponin composition provides a significant protective effect against challenge by wild type *S. equi*. The data also demonstrates that the claimed intranasal *S. equi*/saponin composition provides a significantly enhanced protective effect in horses challenged with wild type *S. equi* over the effect shown by the commercial intramuscular *S. equi* preparation.

17 The claimed intranasal *S. equi* vaccine, the commercial intramuscular vaccine and a control wherein no vaccine was administered were evaluated in three groups of eleven horses. Horses in Groups 1 were vaccinated with the claimed intranasal composition comprising *S. equi* vaccine and 2.5 mg saponin/ml. Horses in Group 2 were vaccinated with the commercial intramuscular vaccine. Horses in Group 3 were not vaccinated and were challenged with a virulent *S. equi* directly. All vaccinates received two vaccinations three weeks apart. All horses were challenged with virulent *S. equi* 21 days post second vaccination. Daily clinical signs indicative of *S. equi* infection were observed up to 35 days post challenge (DPC) from which an average clinical score was calculated.

The results of the study described in the Declaration indicated that:

i) the claimed *S. equi*/saponin intranasal vaccine composition reduced the occurrence of clinical signs of disease in horses, namely ruptured abscesses, by 56% over the occurrence of ruptured abscesses in horses administered the commercial intramuscular vaccine, and the claimed composition reduced the occurrence of ruptured abscesses over the control horses by 51%.

ii) the group of horses vaccinated with the claimed *S. equi*/saponin intranasal vaccine composition showed a 56% reduction in the average clinical score over the horses administered the commercial intramuscular vaccine, and the group of horses vaccinated with the claimed *S. equi*/saponin intranasal vaccine composition showed a reduction in the average clinical score over the control horses of 65%.

18 The data in the Declaration demonstrate that the *S. equi*/saponin intranasal vaccine of the claimed invention provided a significantly enhanced immunostimulatory response compared to unvaccinated horses or the commercial intramuscular *S. equi* vaccine. The horses vaccinated with the claimed composition were significantly protected against clinical disease as compared to the controls following a severe *S. equi* challenge as demonstrated by the decreased incidence of clinical symptoms and a reduction in overall clinical scores.

19 These results demonstrate that it would not be obvious to one of skill in the art that the claimed mucosally administered *S. equi*/saponin vaccine would enhance the immunostimulatory and protective effects of a *S. equi* vaccine by the addition of saponin.

20 In view of the foregoing, applicant respectfully submits that the rejection of the claims under Section 103 cannot stand and respectfully requests that the rejection be withdrawn.

CONCLUSION

In view of the foregoing amendment and comments, applicant submits that the claims are in form for allowance. A notice to that effect is hereby solicited.

Respectfully submitted,

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